Genetic Diversity at the Major Histocompatibility Complex (*B*) and Microsatellite Loci in Three Commercial Broiler Pure Lines[1]

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ABSTRACT Genetic diversity at the MHC and non-MHC loci was investigated in three commercial broiler chicken pure lines. The MHC class II and IV loci were evaluated in Southern hybridizations and molecular genotypes based on RFLP were interpreted from pedigreed families. Four MHC class II and eight class IV genotypes were identified in the broiler lines, and their frequencies differed among the lines. Line-specific MHC genotypes were identified. The observed heterozygosities (59 to 67%) suggest that the MHC loci are highly polymorphic in the broiler lines. At least 9% of the genetic variation at the MHC was due to line differences; the remainder reflected individual variations. To characterize non-MHC genes, 41 microsatellite loci located throughout the chicken genome were evaluated in the broiler lines. Genetic variation was also observed at the microsatellite loci for the broiler lines; the number of alleles at a single locus ranged from one to eight, and the average number of alleles per locus was 3.5, 2.8, and 3.1 for each of the lines, respectively. The observed heterozygosities for microsatellite loci ranged between 0 and 89% in the lines. Based on the fixation index (Fst), about 19% of the genetic variation at microsatellite loci was attributed to broiler line differences. Deviations from Hardy-Weinberg equilibrium were detected at both MHC and non-MHC loci. Possible explanations for these deviations include genetic selection by the primary broiler breeder or the presence of null alleles that were not identified by the typing procedures described in this report. This study contributes to our knowledge on the molecular characteristics and genetic structure of a commercial broiler chicken population. Analysis of MHC and non-MHC loci suggests that there is still sufficient genetic diversity in the broiler lines to continue the progress toward improved broiler chicken production.

(*Key words*: major histocompatibility complex, microsatellite, Hardy-Weinberg, fixation index, genetic distance)

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INTRODUCTION

Tremendous improvements in commercial broiler chicken production have been made through classical breeding approaches. For example, body weight, growth rate, and feed efficiency are a few traits that continue to be improved. In contrast, some traits such as immune response and disease resistance are more difficult to evaluate in breeding populations, and the lack of easily measured phenotypic markers for such traits has limited their application in broiler breeder selection programs.

Immunoresponsiveness and resistance to pathogenic challenge are determined by the individual's genotype and its interaction with the environment. In commercial broiler facilities, all chickens are exposed to similar environmental stimuli of microbial, chemical, or nutritional origin. Therefore, of importance for determining phenotypic variation is the genetics of the chicken because it predetermines the maximum potential of the bird in its environment (Lamont, 1998). Because many immune response and disease resistance measurements are quantitative, it can generally be assumed that the traits are polygenic. Of most importance is the MHC, a polymorphic, multigene system whose products have a primary role in antigen presentation to T lymphocytes during humoral and cell-mediated immune responses. The MHC clearly affects resistance and susceptibility to certain poultry diseases (Shierman et al., 1977; Bacon et al., 1981; Johnson and Edgar, 1986; Lamont et al., 1987a; Heller et al., 1991), as well as immune responses (Bacon et al., 1984; Dun-

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Abbreviation Key: Fst = fixation index; HWE = Hardy-Weinberg equilibrium; MD = Marek's disease; *Rfp*-Y = restriction fragment pattern-Y.

nington et al., 1989; Loudovaris et al., 1990a,b; Yonash et al., 1999a). Similarly, non-MHC genes that are located in various regions of the chicken genome are also associated with these traits (Hu et al., 1997; Vallejo et al., 1997; Xu et al., 1998; Yonash et al., 1999b, 2001). Therefore, characterization of MHC genes and other candidate genetic markers in broiler breeders will be of benefit in selection programs that emphasize immune response and disease resistance.

The genomic organization of the chicken MHC genes is different from that of mammals; the chicken MHC genes have been localized to two independent regions, *B* and restriction fragment pattern-Y (*Rfp*-Y) on chromosome 16 (Bloom et al., 1987; Miller et al., 1994). Both of these regions contain class I (*B-F* or *Y-F*) and class II (*B-L* or *Y-L*) loci; the *B* system encodes classical MHC products, whereas the *Rfp*-Y system was recently suggested to encode nonclassical MHC products (Afanassieff et al., 2001). The chicken *B* system is also in linkage disequilibrium with the class IV (*B-G*) genes whose products serve as the basis for serological typing at the chicken MHC (Guillemot and Auffray, 1989). Recently, Kaufman and coworkers (1999) presented a detailed map of the *B* region. Not only are there class I and class II genes, but also transporter associated with antigen processing (*TAP*) genes, a region of *DM* genes, *RING3*, tapasin, C-type animal lectin-binding genes and the complement factor, C4 gene. Thus, as suggested by Kaufman and coworkers (1995), the chicken *B* region represents a minimal, essential, classical MHC. In contrast, little is known about the class I and II genes of the *Rfp*-Y system. Recent evidence indicates that at least one of the class I loci is relatively polymorphic and it is expressed in a variety of tissues (Afanassieff et al., 2001). In addition, the *Rfp*-Y class I molecules share structural similarities in the antigen-binding region with mammalian nonclassical MHC (class Ib) molecules, and they exhibit low levels of expression similar to the class Ib molecules. Therefore, the MHC genes within the *Rfp*-Y region may represent the homologues of mammalian nonclassical MHC genes. Both the *B* and *Rfp*-Y complexes are polymorphic; at least 30 *B* and several *Rfp*-Y haplotypes have been reported (Dietert et al., 1991; Briles et al., 1993; Miller et al., 1994; Afanassieff et al., 2001).

Over 1,965 DNA-based genetic markers are available for genotyping chickens at non-MHC loci (Groenen et al., 2000). Many of these markers have been mapped to various chromosomes or linkage groups (Levin et al., 1993, 1994; Crooijmans et al., 1994, 1995, 1996a; Cheng et al., 1995; Groenen et al., 1998, 2000). The importance of these DNA-based markers is that they can be in linkage disequilibrium with candidate genes that affect economically important traits. Currently, the chicken linkage map covers about 95% of the entire genome and the average marker interval is less than 20 cM. This accomplishment provides sufficient marker density for mapping candidate genes (Groenen et al., 1998, 2000).

Microsatellites are the primary genetic marker; they are highly polymorphic loci that have been used to map QTL, to estimate genetic variation, to determine parentage, and to determine the phylogeny of organisms (Cheng, 1997; Ortí et al., 1997; Zhou and Lamont, 1999). Microsatellites are composed of tandem repeats of one to six bases and they are variable in length. For example, in chickens, Cheng and Crittenden (1994) reported four to 14 repeat units in "TG" microsatellite loci from the Red Jungle fowl, whereas Crooijmans and coworkers (1993) observed up to 33 repeat units in their "TG" microsatellites that were isolated from a White Leghorn chicken. The variations in length define the alleles at a particular microsatellite locus within a population. The most useful microsatellite markers for gene mapping are those with several length polymorphisms or alleles in the population.

In a companion paper, we reported on the performance of three commercial broiler pure lines for coccidiosis, Marek's disease (MD), and antibody response to SRBC (Emara et al., 2002). Broiler Line 3 was more susceptible to MD and had a lower primary antibody response to SRBC than Lines 1 and 2. In addition, Line 3 birds at an older age (Day 58) were more susceptible to coccidiosis. These phenotypic variations in immune response and disease resistance are probably due to genetic differences among the broiler lines, as well as within-line genetic variation. Therefore, in the current study, we surveyed a random group of individuals from each line to determine the genetic diversity at MHC and non-MHC loci. The allelic composition and frequencies, degree of polymorphism (number of alleles, heterozygosity), genetic distances, and genetic differentiation are presented for each broiler line. The genetic data in this study will provide valuable information that can be used in future experiments to evaluate the role of MHC and non-MHC genes in immune response and disease resistance of commercial broilers.

MATERIALS AND METHODS

Chickens

The commercial broiler pure lines were randomly designated Lines 1, 2, and 3. They have been previously described for their resistance to MD and coccidiosis and their antibody response to SRBC (Emara et al., 2002). In the evaluation of MHC genotypes, all broiler chickens were pedigree-hatched and wing-banded for identification. Parents and progeny from at least 30 dam families, representing 10 sire families were evaluated for their MHC genotypes. For analysis of microsatellite DNA markers, one bird each from 20 distinct sire families (genetically unrelated) was chosen to represent the broiler line, and thus 60 individuals (20/line) were genotyped. Blood samples were collected from all birds for DNA isolation.

Isolation of Genomic DNA

Whole blood was collected in EDTA. Forty microliters of blood was added to 500 μL of SET buffer (0.15 *M* NaCl,

0.05 *M* EDTA, and 0.001 *M* Tris-HCl, pH 7.4) and samples were allowed to digest overnight at 56°C in the presence of 200 μg of proteinase K and 20 μL of 10% SDS. The DNA was extracted twice with phenol-chloroform (1:1, Tris-saturated, pH 8.0) and twice with chloroform-isoamyl alcohol (24:1). The DNA was then precipitated by addition of one-tenth volume of sodium acetate (3 *M*, pH 5.2) and two volumes of 100% ethanol. The DNA pellets were washed once with 70% ethanol, air-dried, and resuspended in TE buffer (10 m*M* Tris-HCl;1 m*M* EDTA).

Chicken B-Region Probes and Labeling

Class II (*B-LβII*) and class IV (*B-G*) probes were used to evaluate the chicken MHC (*B* region). A 2.3-kb *Hind*III fragment that contained the entire chicken *B-LβII* (B^6 haplotype) gene was gel-purified from the genomic clone, CCII-7-1 (Xu et al., 1989). The B-G probe was the *bg* 32.1 cDNA probe described by Miller et al. (1988). Both probes were labeled with ^{32}P-dCTP using a commercial kit[3] and the random priming method (Feinberg and Vogelstein, 1983).

Southern Hybridizations

Genomic DNA (10 μg) was digested with the restriction enzyme, *Pvu*II (3 U/μg of DNA) according to the manufacturer's guidelines. Digested DNA samples were size-separated for 48 h at 36 V in 0.8% agarose gels using 0.5× TBE (0.045 *M* Tris-borate, 0.045 *M* boric acid, and 0.001 *M* EDTA) buffer. The DNA was then subjected to depurination (0.25 *M* HCl), denaturation (1.5 *M* NaCl; 0.5 *M* NaOH), and neutralization (1.5 *M* NaCl, 1.0 *M* Tris-HCl) prior to capillary-transfer in 10× SSC for at least 18 h to Hybond N^+ nylon membranes.[4]

After transfer, the DNA was fixed to the nylon membrane by UV cross-linking in a Stratalinker.[5] Nylon membranes were prehybridized at 65 C for 4 to 5 h with the hybridization buffer (0.263 *M* sodium phosphate, 7% SDS, 1 m*M* EDTA, and 1% BSA). After prehybridization, the membranes were allowed to hybridize overnight at 65 C with the ^{32}P-dCTP labeled probe. Stringency washing conditions for the B-LβII and B-G probes included two 20-min washes at 65 C with each of the following buffers: 2× SSC with 0.1% SDS and then 0.5× SSC with 0.1% SDS. Membranes were subjected to autoradiography with x-ray film and intensifying screens at –70 C.

Microsatellite Genotyping

Forty-one microsatellite primer pairs were randomly chosen from mapping kits 1, 2 and 3 that were kindly provided by Hans Cheng.[6] Amplification of microsatellite markers was conducted using the Touchdown PCR cycling method (Hecker and Roux, 1996). Briefly, the initial annealing temperature for each primer pair was that described in the mapping kits, and the targeted annealing temperature was 5 C lower. The annealing temperature was decreased by 0.5 C for every PCR cycle until the targeted annealing temperature was reached. The PCR conditions were denaturation at 94 C for 2 min, annealing for 30 s with variable temperatures (dependent on the primer pair), and extension at 72 C for 30 s. After 10 PCR cycles, the targeted annealing temperature was reached, and the subsequent 25 cycles were performed at this temperature.

Three PCR products with different fluorescent labels (Fam, Hex, and Tet), as well as the TAMRA 350[7] size marker were mixed together and then, loaded onto a 6% polyacrylamide gel for analysis using the ABI 377[7] automated sequencer. The size of microsatellite alleles was estimated relative to the in-lane Gene Scan TAMRA 350 size standards using Gene Scan and Genotyper software programs.[7]

Statistical Calculations

The frequencies of the MHC RFLP molecular genotypes were estimated from the proportion of homozygotes and heterozygotes within each of the broiler lines; ie., the frequency of an MHC RFLP haplotype = frequency of homozygotes + ½ frequency of heterozygotes. POPGENE Version 1.31 (Yeh et al., 1999) was used to calculate observed and expected heterozygosities [assuming Hardy-Weinberg equilibrium (HWE)], fixation coefficients of subpopulations (lines) within the total population (Fst), and deviations from HWE by maximum likelihood for each microsatellite locus (Yeh et al., 1999). Nei's (1978) unbiased estimates of genetic identity and distance were also calculated using POPGENE Version 1.31.

RESULTS

Upon digestion of genomic DNA with *Pvu*II, four MHC class II (a to d) and eight class IV (A-H) RFLP molecular genotypes were identified in pedigreed families from the three broiler lines (Figures 1A and 2). All MHC RFLP genotypes were observed in homozygous condition in sampled birds, except for the class IV RFLP patterns, B and F. These two genotypes could be differentiated in heterozygotes by genotype-specific *Pvu*II restriction fragments. Variations in composition and frequency of the MHC genotypes were observed in the broiler lines (Figure 1B and Table 1). At least one class II (b) and two class IV (C and G) genotypes were line specific. In addition, some of the class IV genotypes (A, D and F) were high in frequency for one of the broiler lines and low in frequency for the other two lines. Using the RFLP frequency data, it was determined that all three broiler lines were in HWE ($P < 0.05$) at the class II and IV MHC loci, with one exception. Deviations from HWE for the class IV MHC loci were present in Line 3. The average observed hetero-

[3]Promega, Madison, WI.
[4]Amersham, Arlington Heights, IL.
[5]Stratagene, La Jolla, CA.
[6]Poultry Coordinator for the U.S. National Animal Genome Research Program, USDA-ARS-ADOL, East Lansing, MI.
[7]Perkin-Elmer, Applied Biosystems Division, Foster City, CA.

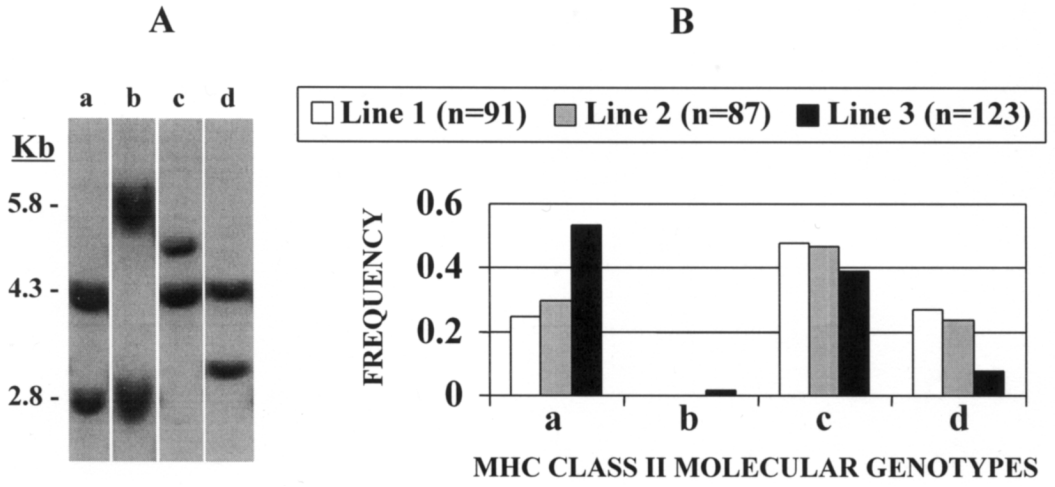

FIGURE 1. MHC class II molecular genotypes and their frequencies among three commercial broiler pure lines. A. Four (a to d) distinct, homozygous RFLP or molecular genotypes were identified among pedigreed broiler chickens using Southern hybridizations and a class II (B-LβII) genomic probe. B. Frequencies of the class II MHC RFLP molecular genotypes were estimated from the proportion of homozygotes and heterozygotes within each of the broiler lines.

zygosity for class II was 0.59, and for class IV, it was 0.67. Calculation of the mean Fst value (0.086) for the MHC loci indicated that about 9% of the genetic variation in the commercial broiler population was due to line differences; the remainder included individual genetic variations.

Forty-one microsatellite loci were evaluated in this study and the total numbers of alleles for these loci were 142, 116, and 127 for Lines 1, 2, and 3, respectively (Table 2). The frequencies of individual microsatellite alleles varied among the broiler lines; these data are not presented, but they can be obtained from the corresponding author upon request. The number of alleles at a single locus ranged from one to eight, with few differences between the broiler lines. The average number of alleles per locus was 3.5, 2.8, and 3.1 for Lines 1, 2, and 3, respectively. Line-specific alleles were observed for 32 of the 41 markers. The degree of polymorphism, based on observed heterozygosity (H) varied with the marker and line (Table 2). The highest polymorphism (H = 0.89) was observed with marker MCW094 on E52 in Line 3. In contrast, several microsatellite loci were monomorphic (H = 0) for one or more of the broiler lines. The Fst coefficients for the microsatellite markers showed large variation, ranging from 0.017 (ADL037) to 0.487 (ADL265), as shown in Table 2. The mean of these Fst estimates indicated that about 19% of the total genetic variation in the broiler population could be explained by line differences. The remaining genetic variation is attributed to differences among individuals. Discrepancies between the observed and expected heterozygosities are also reflected in significant deviations from HWE for several of the microsatellite loci (Table 2). The deviations from HWE varied with the microsatellite marker and broiler line. At least 26 loci

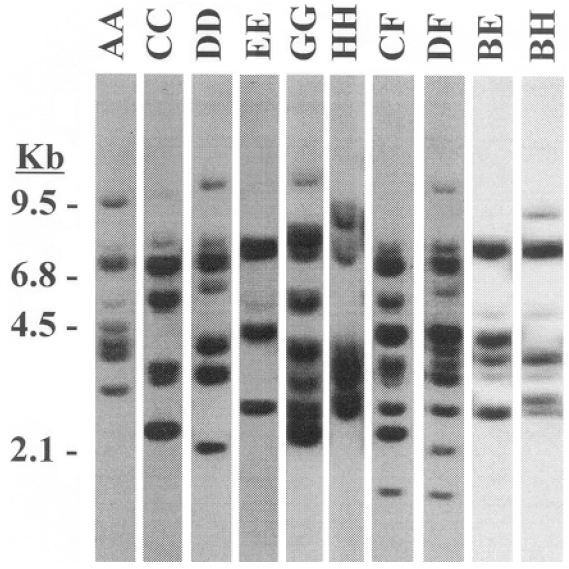

FIGURE 2. MHC class IV molecular genotypes among three commercial broiler pure lines. At least eight molecular genotypes (A to H) were identified in pedigreed broiler families using Southern hybridizations and a *B-G* cDNA probe (Bg32.1). Two molecular genotypes (B and F) were not observed in homozygous condition among the broiler chickens that were examined; they are shown as heterozygotes.

TABLE 1. Frequencies of the MHC class IV molecular genotypes among three commercial broiler pure lines

	A[1]	B	C	D
Line 1	0.01	0.01	0	0.03
Line 2	0	0	0	0
Line 3	0.27	0.01	0.40	0.20
	E	F	G	H
Line 1	0	0.62	0	0.33
Line 2	0.24	0	0.45	0.31
Line 3	0.10	0.02	0	0

[1]The MHC class IV molecular genotypes (A to H) are the same as those shown in Figure 2.

TABLE 2. Number of alleles per locus, observed and expected heterozygosities (H), F-statistics (Fst), and Hardy-Weinberg equilibrium (HWE) for 41 microsatellite loci within three commercial broiler pure lines (L1, L2, and L3)[1]

Microsatellite locus	LG[2]	Alleles/Locus (n)			H (Observed)			H (Expected)[3]			Fst[3]	HWE[3]		
		L1	L2	L3	L1	L2	L3	L1	L2	L3		L1	L2	L3
MCW 168	1	3	2	2	0.16	0.70	0.30	0.23	0.46	0.32	0.107	NS	*	NS
MCW010	1	2	2	2	0.45	0.53	0.25	0.40	0.39	0.22	0.327	NS	NS	NS
ADL124	1	3	3	4	0.44	0.47	0.42	0.64	0.48	0.69	0.155	*	NS	**
ADL150	1	2	1	3	0.05	0	0.50	0.05	0	0.56	0.309	NS	NS	NS
MCW068	1	6	4	5	0.36	0.50	0.67	0.70	0.56	0.63	0.083	**	***	NS
ADL037	1	1	1	2	0	0	0.05	0	0	0.05	0.017	NS	NS	NS
MCW023	1	3	2	3	0.28	0.24	0.75	0.61	0.29	0.64	0.113	*	NS	NS
ADL101	1	4	1	2	0.37	0	0.11	0.66	0	0.10	0.263	*	NS	NS
MCW082	2	1	2	1	0	0.10	0	0	0.32	0	0.143	NS	***	NS
ADL176	2	3	4	3	0.75	0.37	0.42	0.65	0.35	0.59	0.223	NS	NS	NS
ADL235	2	1	2	1	0	0.35	0	0	0.29	0	0.124	NS	NS	NS
ADL300	2	6	4	7	0.47	0.22	0.40	0.57	0.25	0.76	0.101	NS	NS	***
ADL157	2	3	3	3	0.10	0	0.45	0.45	0.64	0.46	0.190	***	***	NS
MCW056	2	4	4	4	0.78	0.83	0.32	0.68	0.73	0.63	0.065	NS	NS	**
LEI104	2	2	3	1	0	0.12	0	0.36	0.53	0	0.307	***	***	NS
ADL177	3	3	2	2	0.26	0.45	0.70	0.23	0.50	0.50	0.129	NS	NS	NS
ADL155	3	2	2	2	0.10	0.45	0.30	0.10	0.44	0.26	0.364	NS	NS	NS
ADL306	3	5	4	4	0.80	0.65	0.75	0.77	0.69	0.68	0.043	NS	NS	NS
ADL237	3	5	2	3	0.59	0.58	0.58	0.56	0.48	0.43	0.131	NS	NS	NS
ADL317	4	3	4	3	0.40	0.65	0.30	0.59	0.56	0.49	0.026	*	NS	NS
ADL145	4	6	5	4	0.80	0.68	0.55	0.74	0.67	0.60	0.166	NS	NS	***
ADL246	4	4	4	4	0.65	0.47	0.20	0.67	0.52	0.40	0.229	NS	NS	***
ADL265	4	4	4	3	0.30	0.26	0.55	0.27	0.24	0.44	0.487	NS	NS	NS
ADL247	5	4	2	2	0.55	0.42	0.25	0.44	0.47	0.29	0.086	NS	NS	NS
MCW038	5	5	5	3	0.20	0.50	0.36	0.47	0.62	0.53	0.140	***	NS	NS
MCW029	5	4	6	5	0.35	0.26	0.32	0.56	0.76	0.78	0.085	***	***	***
ADL142	6	3	3	3	0.52	0.67	0.67	0.52	0.54	0.66	0.082	NS	NS	NS
ADL244	6	4	3	3	0.47	0.06	0.22	0.65	0.52	0.51	0.271	NS	***	*
ADL169	7	3	2	5	0.11	0.10	0.39	0.27	0.10	0.52	0.470	**	NS	NS
ADL111	7	3	2	4	0.30	0	0.65	0.43	0.10	0.69	0.201	NS	***	NS
ADL302	8	2	2	4	0.50	0.05	0.63	0.42	0.05	0.63	0.166	NS	NS	***
ADL117	Z	2	2	2	0.25	0.10	0.15	0.47	0.10	0.35	0.366	*	NS	**
ADL201	Z	2	2	2	0	0.05	0.10	0.18	0.05	0.26	0.032	***	NS	**
MCW035		3	1	2	0.56	0	0.35	0.53	0	0.35	0.141	NS	NS	NS
ADL289	E27	2	1	3	0.30	0	0.55	0.48	0	0.43	0.156	NS	NS	NS
ADL102	E29	7	3	4	0.50	0.22	0.10	0.70	0.59	0.48	0.207	***	**	***
ADL272	E29	3	2	3	0.50	0.22	0.47	0.45	0.28	0.59	0.095	NS	NS	NS
ADL210	E30	3	3	3	0.40	0.11	0.11	0.55	0.28	0.28	0.351	NS	***	***
MCW134	E36	5	2	3	0.37	0.07	0	0.75	0.07	0.40	0.204	**	NS	***
ADL293	E41	3	2	4	0.06	0.05	0.30	0.17	0.05	0.66	0.424	***	NS	***
MCW094	E52	8	7	4	0.40	0.68	0.89	0.82	0.80	0.72	0.096	***	***	NS
Mean		3.5	2.8	3.1	0.35	0.30	0.37	0.46	0.36	0.45	0.189			

[1]Twenty individuals were randomly-selected and genotyped from each broiler line.

[2]Linkage group (LG) or chromosomal location of microsatellite loci.

[3]POPGENE Version 1.31 (Yeh et al., 1999) was used to calculate expected heterozygosity based on Nei's formula (1973), Fst values (Hartl and Clark, 1997), and chi-squared analysis for Hardy-Weinberg equilibrium (HWE). Statistical significance was recorded at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

showed deviations ($P < 0.05$) from HWE in the broiler lines.

Based on the microsatellite genotyping data, the genetic identity and distance between the broiler lines indicated that Lines 2 and 3 were most identical, with the shortest genetic distance (0.2161) between them (Table 3). Line 1 was genetically distinct, especially from Line 2. Interestingly, estimation of the genetic distance using the MHC class II genotyping data indicated that Line 1 was more closely related to Line 3 (distance = 0.1073) than to Line 2 (distance = 0.1307). In contrast to the microsatellite data, the MHC data suggested that Lines 2 and 3 were the most distantly related with a value of 0.2522.

DISCUSSION

The results demonstrate that the commercial broiler chicken lines in this study exhibit a moderate level of

TABLE 3. Genetic identity and genetic distance among three commercial broiler lines (L1, L2, and L3)[1]

	L1	L2	L3
L1	—	0.6622	0.7920
L2	0.4121	—	0.8057
L3	0.2332	0.2161	—

[1]Genetic identity (above the diagonal) and genetic distance (below the diagonal) were calculated from the microsatellite allele frequency data according to Nei's unbiased estimates (1978).

genetic diversity at both MHC and non-MHC (microsatellite) loci. The genetic divergence may be due to differences in the ancestry of these broiler lines or alternatively, it may be due to artificial selection practices. The broiler lines are maintained as closed populations and they are under intensive selection for various growth and reproductive traits. Evidence has accumulated to indicate that selection practices alter the genetic composition of chicken populations (Gavora et al., 1986; Lamont et al., 1987b; Dunnington et al., 1990). For instance, long-term divergent selection (31 generations) for 8-wk body weight in chickens resulted in two populations with line-specific minisatellite DNA bands (Dunnington et al., 1990, 1994). In fact, 48% of the DNA fingerprint bands were line-specific. Therefore, long-term selection had resulted in the fixation of certain alleles or DNA fragments within each of these experimental lines.

In the current study, genetic variation was most notable at the MHC loci, where composition and frequency of the genotypes differed among the broiler lines. Based on Fst, the genetic variation (9%) at the MHC (class II) in the broiler population was only partially due to line differences. The remainder is attributed to differences between individuals within each line; therefore, within-line selection for immune response and disease resistance is a possibility for this broiler population. The three broiler lines shared some MHC class II and IV genotypes (based on RFLP patterns); however, differences in their composition and frequencies were present. The differences at the MHC may be due to ancestry or alternatively, correlations to other traits under selection within a line and indirect selection for certain MHC genotypes. It should be noted that the MHC loci were in HWE, with one exception at the class IV loci (Line 3). Therefore, with Line 3, at least indirect selection or some other genetic force seems to be affecting the MHC class IV loci.

At least one MHC class II genotype (b) was line-specific, although at low frequency. Line-specific class IV genotypes were also observed. The MHC class IV loci were more highly polymorphic in the broiler lines than the class II loci, as demonstrated by the greater number of RFLP genotypes that were identified and the higher observed heterozygosity. Similar to previous findings, some class II and IV genotypes in the broilers were in strong linkage disequilibrium in pedigreed families, thus defining specific MHC haplotypes (Chaussé et al., 1989; Warner et al., 1989; Lamont et al., 1990; Li et al., 1999).

Reports that describe MHC haplotypes in meat-type or broiler chickens are increasing (Heller et al., 1991; Uni et al., 1992, 1993; Landesman et al., 1993; Li et al., 1997; 1999; Zheng et al., 1999; Livant et al., 2001). Although some of the molecular MHC genotypes in this study are similar to previously reported RFLP genotypes in broiler chickens (Uni et al., 1992, 1993; Landesman et al., 1993; Li et al., 1997; 1999; Zheng et al., 1999; Livant et al., 2001), it is difficult to determine the precise identity without comparative DNA sequence analyses of the MHC genes. However, based on the findings of Li and coworkers (1997, 1999) who showed that some of the broiler MHC haplotypes were similar to standard chicken MHC haplotypes, it is expected that the different broiler chicken populations will also have MHC genes and haplotypes in common. In addition, it is also expected that other rare MHC genotypes or alleles in the broiler lines were not identified in these experiments due to the sampling size. Usually, rare alleles are maintained at frequencies less than 0.005 in the population by recurrent mutation at the genetic locus (Hartl and Clark, 1997). The importance of these rare alleles is not known; however, they are important contributors to genetic diversity. Thus, continued characterization of genetic polymorphisms in the chicken MHC genes is warranted.

Genotyping at various microsatellite loci across the chicken genome also indicated that the broiler lines were genetically different. The microsatellite allelic composition and frequencies differed among the three broiler pure lines, and some alleles were observed as line-specific. Several alleles were also shared among the broiler lines; however, caution is warranted because previous researchers have demonstrated that although certain microsatellite alleles were the same size, their flanking DNA sequences were different (Garza and Freimer, 1996; Ortí et al., 1997). The high mutation rates of microsatellite loci usually result in alleles of the same size (size homoplasy) at a locus; however, the flanking sequences can be very different, especially across populations (Garza and Freimer, 1996). Further characterization of similar-sized microsatellite alleles by DNA sequencing would be needed to confirm that the alleles from separate broiler lines are identical by descent.

In chickens, the number of alleles per microsatellite locus in any one population has ranged from one (monomorphic) up to several alleles (Crooijmans et al., 1993, 1996b; Cheng et al., 1995). For instance, Crooijmans and coworkers (1996b) examined 17 microsatellite markers in nine broiler and six layer lines. Interestingly, they found that the average number of alleles at the loci was higher in broilers (5.2 alleles) than in layers (3.0 alleles) and the degree of heterozygosity among the microsatellite loci was higher in broilers (53%) than in layers (27%). Similar to Crooijmans and coworkers (1996b) who reported an average of 3.6 alleles per marker in broiler lines and to Kaiser and coworkers (2000) who reported 2.8 and 2.9 alleles per marker in two broiler populations, we observed a range of 2.8 to 3.5 alleles per marker in the broiler lines. However, in our study, the observed heterozygosity in the broiler lines (range = 30 to 37%) was much lower than the heterozygosity (53%) reported by Crooijmans and coworkers (1996b). These findings may be due to differences in population structure or the differences may be due to sampling size. In their studies, Crooijmans and coworkers (1996b) evaluated 60 individuals per line and 17 microsatellite markers, whereas we evaluated 41 markers and 20 individuals per line.

Departures from HWE were observed for several microsatellite markers in the broiler lines and they were often associated with decreased observed heterozygosities compared to the expected values. This observation

may be the result of several possibilities. Because the broiler lines are maintained separately and selected for independent traits, one reason for the HWE deviations is genetic selection. Some of these microsatellite markers may be linked to economically important genes that may be under intensive selection within the specific broiler line. Fixation of alleles and thus, an increase in homozygosity (or decrease in heterozygosity) is expected to occur in these highly selected lines. Departures from HWE may also be due to the presence of null alleles that were not amplified during the PCR; null alleles would result in an overestimate of homozygotes. Lastly, the small sample sizes and genotyping error may also be factors to explain the deviations from HWE.

In our studies, an average of 19% of the genetic differentiation at microsatellite loci was due to line differences. However, the amount of genetic differentiation was dependent on the microsatellite locus, and there was substantial differences (17 to 49%) among the loci. As suggested by Wright (1978), Fst values between 5 to 15% are considered moderate genetic differentiation; between 15 to 25%, significant; and between 25 to 50%, very significant. The Fst value is a reflection of the inbreeding-like effects at a locus and perhaps, some of the loci with moderate to high Fst values may identify chromosomal regions that are approaching fixation, as the result of genetic selection in the broiler lines.

The relative distance between lines or populations may be due to differences in ancestry, genetic selection, gene flow, genetic drift or genetic bottlenecks. Because the broiler lines are closed and independent, ancestry and genetic selection are probably the main contributors to the genetic divergence. The amount of genetic distance between the broiler lines (0.2161 to 0.4121) is comparable to distances reported for sheep (0.079 to 0.502) and human (0.005 to 0.355) populations (Buchanan et al., 1994; Deka et al., 1995). It was not surprising to find that the genetic relationships of the three broiler lines were dependent on the genetic locus that was evaluated (Russo et al., 1996). Because the MHC data are from one chromosome (no. 16) and the microsatellite data are more representative of the genome, it is suspected that the genetic distances calculated from the microsatellite data reflect the true nature of the relationships between the broiler lines.

This study contributes to our knowledge on the molecular genetic characteristics and genetic structure of a commercial broiler chicken population. The data suggest that there is still sufficient genetic diversity in the broiler lines to continue the progress toward improved broiler chicken production. Identification of line-specific alleles will be useful in pedigree identification. The broiler lines in this study were demonstrated to be phenotypically different in immune response and disease resistant traits (Emara et al., 2002). The portion of this phenotypic variation that is due to the genotype, and the actual candidate genes that influence these immune response and disease-related traits are not known. As indicated by Hillel (1997), a resource population for mapping QTL or genes of economic importance should originate from genetically distinct chicken lines or populations. Therefore, the genetic data provided in this study will be useful in the development of resource populations to evaluate QTL. In addition, with the increased focus on genetic conservation, the identification of chicken populations or lines that have unique alleles may be of use in decisions to maintain such birds. These decisions will be of importance, especially if the alleles are associated with economically important traits.

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